Meadow plant growth and competition under elevated ozone and carbon dioxide

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Academic dissertation

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This thesis is based on the following articles and manuscripts, which are referred to in the text by their Roman numerals:

I Rämö, K., Aikio, S., Manninen, S. Light, nutrients and competition in the growth and biomass allocation of *Agrostis capillaris* and *Ranunculus acris* – Manuscript under revision.

II Rämö, K., Aikio, S., Kanerva, T., Ojanperä, K., Manninen, S. Are plant-plant interactions modified by elevated ozone and carbon dioxide? – Manuscript.

III Rämö, K., Kanerva, T., Nikula, S., Ojanperä, K., Manninen, S. Influences of elevated ozone and carbon dioxide in growth responses of lowland hay meadow mesocosms – Environmental Pollution, *in press*.

IV Rämö, K., Kanerva, T., Ojanperä, K., Manninen, S. Growth onset, senescence, and reproductive development of meadow species in mesocosms exposed to elevated O_3 and CO_2 – Environmental Pollution, *in press*.

V Rämö, K., Slotte, H., Kanerva, T., Ojanperä, K., Manninen, S. Growth and visible injuries of four *Centaurea jacea* L. ecotypes exposed to elevated ozone and carbon dioxide – Environmental and Experimental Botany, *in press*.

Contributions

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Abstract

The main aim of my thesis project was to assess the impact of elevated ozone (O_3) and carbon dioxide (CO_2) on the growth, competition and community of meadow plants in northern Europe. The thesis project consisted of three separate O_3 and CO_2 exposure experiments that were conducted as open-top-chamber (OTC) studies at Jokioinen, SW Finland, and a smaller-scale experiment with different availabilities of resources in greenhouses in Helsinki. The OTC experiments included a competition experiment with two- and three-wise interactions, a mesocosm-scale meadow community with a large number of species, and a pot experiment that assessed intraspecific differences of *Centaurea jacea* ecotypes. The studied lowland hay meadow proved to be an O_3-sensitive biotope, as the O_3 concentrations used (40-50 ppb) were moderate, and yet, six out of nine species (*Campanula rotundifolia, Centaurea jacea, Fragaria vesca, Ranunculus acris, Trifolium medium, Vicia cracca*) showed either significant reductions in biomass or reproductive development, visible O_3 injury or any two as a response to elevated O_3. The plant species and ecotypes exhibited large intra- and interspecific variation in their response to O_3, but O_3 and CO_2 concentrations did not cause changes in their interspecific competition or in community composition. However, the largest O_3-induced growth reductions were seen in the least abundant species (*C. rotundifolia* and *F. vesca*), which may indicate O_3-induced suppression of weak competitors. The overall effects of CO_2 were relatively small and mainly restricted to individual species and several measured variables. Based on the present studies, most of the deleterious effects of tropospheric O_3 are not diminished by a moderate increase in CO_2 under low N availability, and variation exists between different species and variables. The present study indicates that the growth of several herb species decreases with increasing atmospheric O_3 concentrations, and that these changes may pose a threat to the biodiversity of meadows. Ozone-induced reductions in the total community biomass production and N pool are likely to have important consequences for the nutrient cycling of the ecosystem.

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SUMMARY

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Introduction

1. Natural factors affecting plant growth

Plant growth requires carbon dioxide (CO_2), water, nutrients and the energy of solar radiation. The availability of material resources and energy to plants is determined by the interaction between abiotic and biotic factors. Abiotic factors are constraints of resource gain and growth, but the amount of resources attained by an individual plant is determined by the presence, species and size of neighboring plants (Tilman, 1988). For instance, nutrients and water have intrinsic dynamics determined by, e.g., the amount of precipitation and the characteristics of soil parent material and vegetation history, but the amount that an individual plant receives is determined by competition. Similarly, temperature and light that are mainly determined by latitude, exposition, cloudiness and other climatic factors (Larcher, 2003), are further modified by the canopy structure. In addition, different functional groups, plant species and ecotypes have inherent differences in growth rates and maximum sizes (Crawley, 1997), as the different life history traits and growth strategies are adjusted to particular biotopes.

Plant growth is strongly determined by the presence of other individuals, be they of the same or different species. By strict definition, competition is considered to be negative interaction resulting from mutual use of shared resources, but the definition can be widened to include the whole shared environment, including pollinators, herbivores, etc. (Goldberg, 1990). In general, plant-plant interactions are negative and result in the reduction of the fitness of one or all of the competitors (Begon et al., 1996). However, the ability of leguminous plants to form symbiosis with nitrogen (N_2) fixating bacteria may lead to partly positive plant-plant interactions. Legumes provide much of their own N through atmospheric N_2 fixation by symbiotic bacteria, which may reduce competition between legumes and other species in question (Mulder et al., 2002). Alternatively, legumes may increase the soil N concentration through leaching and decomposition of N-rich detritus (Mallarino and Wedin, 1990; Rannells and Wagger, 1997), with consequences for competition among non-leguminous species (Connell, 1990).

As plant growth is dependent on the availability of resources, plants try to maximize their growth by allocating biomass to the organ that is responsible for acquiring the most limiting resource (Aikio and Markkola, 2002; Bloom et al., 1985; Tilman, 1988). This so-called optimal resource use theory predicts that plants should increase allocation to shoots when light is more limiting than nutrients, and to roots when nutrients and water are more limiting than light. The theory can be extended to predict the effects of gases such as ozone (O_3) and CO_2. Ozone has the capacity to damage the foliage of plants, in which case more growth is allocated to repair processes and thus less is allocated to the roots (Cooley and Manning, 1987). In response to elevated CO_2, plants try to allocate growth to roots as nutrients become more limiting (Bazzaz, 1990).

2. Plant responses to climate change

The growth of plants is also modified by human influence. Since the invention of fire, humans have modified earth's vegetation in countless ways, and at present there are hardly any areas where humans' fingerprints are absent. This is because in addition to changes in land use (e.g. forestry, agriculture and urbanization), humans modify the atmosphere, including emissions of greenhouse gases. Since the industrial revolution, the concentrations of the greenhouse gases [e.g. CO_2, methane (CH_4), nitrous oxide (N_2O) and tropospheric O_3] have increased dramatically, mainly due to combustion of fossil fuels and land-use changes. Although the natural greenhouse effect enables life on earth in its present form, prolific increases in greenhouse gas concentrations may lead to changes that pose severe threats to ecosystems and humans themselves (IPCC, 2001). The projections for the current century predict, for instance, increases in surface temperatures, changes in the sea level and precipitation, and increased occurrence of extreme weather events (IPCC, 2001). Moderate increases in temperature and precipitation may, however, initially enhance plant growth (Crawley, 1997).

Anthropogenic emissions and changes in the atmosphere have varying interacting direct and indirect effects, and to truly understand and estimate possible future changes in the structure and functioning of ecosystems, combined studies on multiple stresses are needed. For instance, moderate increases in temperature generally enhance CO_2-driven stimulation of photosynthesis in C3 species (Poorter and Pérez-Soba, 2001; Runeckles, 2002), but this stimulation may be reduced by elevated tropospheric O_3. In this thesis I have concentrated on addressing the concomitant effects of two greenhouse gases, O_3 and CO_2 that have mainly opposing direct effects on plant growth.

2.1. Tropospheric ozone (O_3)

Ozone is not emitted as such, but it is formed under sunlight in a fairly simple series of reactions from precursor pollutants: nitrogen oxides (NO_x), oxygen (O_2), carbon monoxide (CO) and volatile organic compounds (VOCs). Ozone occurs naturally in the earth's troposphere, but human actions, namely combustion of fossil fuels, have increased the levels markedly. Pre-industrial O_3 concentrations are thought to have averaged around 10 - 20 ppb (parts per billion), and at present background O_3 concentrations are rising at a rate of approximately 0.5 - 2% per year (Vingarzan, 2004). Current background O_3 concentrations in the Northern Hemisphere range between 20 and 45 ppb (Vingarzan, 2004). Ozone is a regional air pollutant, as elevated O_3 concentrations can be measured at great distances from the precursor sources. However, because of the duplex nature of O_3 formation and scattering (Kley et al., 1999), the highest concentrations can be found in rural sites surrounding metropolitan areas. Ozone concentrations in Southern Finland are generally low compared with the concentrations measured in the densely inhabited areas of Central and Southern Europe and the United States. Daytime averages in May-July in Southern Finland range around 40 ppb, and they are influenced by the long-range transport of O_3 and precursors from Central Europe (Laurila et al., 2004). Within vegetation the concentrations of O_3 typically form a vertical gradient due to O_3 uptake and destruction at plant surfaces, resulting in a significant decrease in O_3 concentrations from the top to the bottom of the canopy (Davison et al., 2003; Finkelstein et al., 2004; Fuhrer et al., 2005).

The direct effects of O_3 on plants are mainly negative, and it is considered the major phytotoxic air pollutant in Europe. Ozone is taken via stomata during the uptake of CO_2 and loss of water (Reich, 1987). Inside the leaf O_3 forms free radicals and oxidizes membranes (Kangasjärvi et al., 1994; Pell et al., 1997; Polle, 1998), and if protection mechanisms fail to repair the damage, plants may experience a wide range of physiological changes, such as alterations in membranes and stomatal functioning, and a decline in photosynthetic capacity (Runeckles and Chevone, 1992). At the plant level, O_3 symptoms may be exhibited as visible injuries or enhanced senescence (Davison and Barnes, 1998; Mills et al., 2005), reductions in the growth rate, and changes in resource allocation and reproductive performance (Chappelka, 2002; Lyons and Barnes, 1998; Pearson et al. 1996; Reiling and Davidson, 1992), i.e. they have serious consequences for the absolute fitness of the plant. Different O_3 injuries do not, however, necessarily correlate with each other (Bergmann et al., 1995; Pleijel and Danielsson, 1997), and the effects of O_3 can be strongly dependent on the measured variables and species in question (Pearsson et al., 1996). The effects of O_3 have predominantly been studied with crops and trees, and studies on wild plants have only begun to appear (Black et al. 2000; Davison and Barnes, 1998; Fuhrer et al., 2005).

The effects of O_3 are reliant on numerous abiotic and biotic factors. For instance, length of day (availability of light), temperature, relative humidity and soil moisture and nutrient status may modify the effects of O_3, as they are important determinants of gas uptake and growth rate (Ashmore and Ainsworth, 1995; Bungener et al., 1999a,b; Davison and Barnes, 1998; DeTemmerman et al., 2002; Fuhrer et al., 2005; Heagle et al., 1989). Furthermore, species differ in their responses to O_3, and considerable variation exists even within wild species (e.g. Bassin et al., 2004; Franzaring et al., 2000; Fuhrer et al. 2005; Nebel and Fuhrer, 1994). Due to intra- and interspecies differences in sensitivity, O_3 is not equally phytotoxic to all individuals. The characteristics making taxa sensitive to O_3 are still under debate, but high stomatal conductance, a high relative growth rate and fast (reproductive) development are among those suggested (Bassin et al., 2004; Bungener et al., 1999a&b; Franzaring et al., 2000; Nebel and Fuhrer, 1994). Although there is no consensus on whether O_3 sensitivity can be linked to different functional groups, leguminous species are repeatedly among the most sensitive to O_3 (Fuhrer et al., 2005). As studies were initially conducted on crops and forest trees, experiments dealing with O_3 were performed on singly grown plants or monocultures (Davison and Barnes, 1998), which was purposeful in the case of crops that are typically grown as monocultures at certain densities. The situation may be quite different for wild species that grow under lower human impact and naturally under strong competition for resources.

Competition may modify the canopy structure and the availability of light and nutrients – all factors that may alter species responses to O_3 (Davison et al., 2003; Finkelstein et al., 2004; Fuhrer et al., 2005). For instance, the availability of light controls stomatal conductance, which influences O_3 uptake. On the other hand, the density of the canopy determines the O_3 concentrations within the canopy and consequently the O_3 dose per individual plant (Finkelstein et al., 2004). Studies on varying nutrient and O_3 levels of wild plants are scarce, but several studies on singly grown plants have shown that low availability of nutrients may increase a species' sensitivity to O_3 (Fuhrer et al., 2005; Pääkkönen et al., 1995; Whitfield et al., 1998). In the light of this, the effects of O_3 on plant species mixtures should strongly depend on the characteristics of the species involved, as shown by Nussbaum et al. (2000).

Previous studies have reported a variety of plant responses to O_3 under competition. Competition may decrease (Barbo et al., 1998; 2002), increase (Andersen et al., 2001; Gimeno et al., 2004, Scebba et al., 2006) or have no effect (Tonneijck et al., 2004) on the O_3 sensitivity of studied species. The first O_3 studies that included interspecific competition were conducted on economically important grass/clover mixtures (e.g. Fuhrer et al., 1994; Pleijel et al., 1996; Rebbeck et al., 1988; Wilbourn et al. 1995), but recent experiments have involved a wider spectrum of species (Andersen et al., 2001; Danielsson and Pleijel, 1999; Gimeno et al., 2004; Nussbaum et al., 2000). The results show that O_3 may alter the balance between clover and grasses, so that more sensitive clover declines relative to grasses at higher O_3 concentrations

(Fuhrer et al., 1994; Nussbaum et al., 1995). In more complex grassland communities, the fraction of grasses has been found to increase at the expense of legumes with increasing O_3 concentrations (Ashmore and Ainsworth, 1995; Ashmore et al., 1995; Fuhrer et al., 2005; Volk et al., 2006). Recent multiyear studies by Tonneijck et al. (2004) and Bender et al. (2006), however, showed that elevated O_3 did not induce changes in either relative or absolute biomass when plants were grown in either monocultures or species mixtures. Changes in species dominance, especially reductions in the proportion of N-fixing legumes, might have a critical role in the nutrient balance of the communities and have significant feedback effects on the whole community. Most of the O_3 experiments with wild plants have been conducted in relatively small pots in OTCs, whereas studies on more natural ground-planted or intact communities under free-air fumigations such as one by Volk et al. (2006) are almost entirely lacking.

Because the concentrations of tropospheric O_3 are increasing due to human activities, international efforts have been established to restrict emissions of O_3 precursor pollutants. Among them are the UN-ECE critical levels for tropospheric O_3, which are widely used in European legislation and air pollution abatement. The current critical level for protecting semi-natural vegetation as proposed by the UN-ECE convention is an AOT40 (accumulated exposure over threshold of 40 ppb) value of 3000 ppb h over a three-month period (Karlsson et al., 2003). The value, however, was recently revised in Obergurgl, Austria, and the provisional recommendation for the next AOT40 value is 5000 ppb h over a six-month period (M. Ashmore, personal communication).

2.2. Carbon dioxide (CO_2) alone and as a mitigator of O_3 stress

Carbon dioxide is the most abundant human-emitted greenhouse gas. The concentrations of CO_2 have risen from the pre-industrial 280 ppm to the current 368 ppm, and the concentrations are projected to range between 540 and 970 ppm by the end of this century (IPCC, 2001). The direct effects of CO_2 have been studied relatively widely (Ainsworth and Long, 2005; Bazzaz, 1990; Bazzaz and McConnaughay, 1992), and in this thesis I have mostly concentrated on assessing the role of CO_2 as a mitigator of O_3 damage.

The direct effects of CO_2 are mainly beneficial, including improved water use efficiency, stimulation of photosynthesis, growth, and enhanced resource allocation to roots (Bazzaz, 1990; Jablonski et al., 2002), although these responses vary with plant species, growth stage and environmental conditions (Bazzaz, 1990; Leadley et al., 1999). Carbon dioxide is reported to enhance overall plant development and senescence in several species (Rogers et al., 1994). Several studies have reported that CO_2 accelerates flowering and increases flower and fruit weight (e.g. Bazzaz, 1990; Deng et al., 1998), but a review by Jablonski et al. (2002) showed that

these changes are species-specific. Plants do not compete directly for CO_2, but it can indirectly modify plant-plant interactions by intensifying competition for light, water and nutrients (Bazzaz and McConnaughay, 1992; Leadley et al., 1999). Contrary to O_3 responses, the proportion of legumes and other forbs has been seen to increase when grown in competition with grasses in a CO_2-enriched environment (Owensby et al., 1993, 1999; Schenk et al., 1997; Warwick et al., 1998), although species- and genera-specific differences in responsiveness exist (Leadley et al., 1999).

The responses of wild plants to a combination of elevated O_3 and CO_2 are largely unstudied and controversial (Johnson et al., 1996; Mortensen, 1997), but studies of crops suggest that elevated CO_2 concentrations often completely or partially prevent or delay the deleterious effects of O_3 on growth (Allen, 1990; Booker et al., 2005; Fiscus et al., 1997; McKee et al., 1997; Morgan et al., 2003; Polle and Pell, 1999). However, a limited number of studies also report no amelioration by CO_2 (Balaguer et al., 1995; Barnes et al., 1995; Heagle et al., 2002, 2003), and especially the responses of yield components remain insufficiently studied. The mechanisms underlying amelioration are not fully understood, but several explanations have been suggested. Elevated concentrations of CO_2 may lower O_3 flux into leaves (Allen, 1990; Cardoso-Vilhena et al., 2004; Fiscus et al., 1997; McKee et al., 2000), and increase the availability of photosynthates that can be used for detoxification and repair processes (Allen, 1990; Polle and Pell, 1999). Ozone - CO_2 interactions can also be viewed the other way: phytotoxic O_3 reducing the stimulating (fertilization) effect of CO_2.

Aims of the thesis

The main aim of my thesis project was to assess the impact of elevated O_3 and CO_2 on the growth, competition and community of meadow plants in northern Europe (**II-V**; Fig. 1). At the onset of my thesis project, information on the effects of O_3 on wild plants was scarce (Black et al., 2000; Davison and Barnes, 1998), and studies on crops suggested that the climatic conditions in Northern Europe (long days and high relative humidity) may increase O_3 uptake, thus making plants (and communities) more susceptible to O_3 damage (Benton et al., 2000; De Temmerman et al., 2002; Embersson et al., 2000; Pleijel et al., 1999). Additionally, the role of O_3 in interspecific competition was relatively little addressed (Davison and Barnes, 1998), but the existing data suggested that it might be an important factor that shapes wild plant communities (e.g. Barbo et al., 1998, 2002). The role of CO_2 as a mitigator of O_3 damage had and has mainly been assessed in relation to crops and forest trees, and therefore it was a topic of high interest with regard to

wild plants (**II-V**). The present combined study of multiple stresses was intended to provide information on the fate of meadow communities under the future atmosphere.

Specifically, I wanted to learn what the effects of O_3 are on individual species and ecotypes, and whether there is intra- and interspecific variation within these responses. Furthermore, I was interested in seeing if these differences in O_3 sensitivity would translate to changes in interspecific competition. I wanted to observe whether O_3 and CO_2 alter the species performance and community structure of a lowland hay meadow. Ultimately, I aimed to fill some of the gaps in knowledge and provide more information on the sensitivity of Northern European wild plants and a particular community lowland hay meadow.

In addition to applied research, I aimed to address questions of basic research by obtaining more in-depth information on the competitive interactions (**I-II**) and resource allocation of several selected species (**I**).

The more specific hypotheses were:

- Species of lowland hay meadows are sensitive to O_3, which can be seen as, e.g., visible O_3 injuries, and reductions in growth and reproduction (**II-V**).
- Plants species and ecotypes vary in their response to O_3 and CO_2. The most responsive group are legumes (**II-V**).
- Different types of O_3 injury do not correlate with each other (**II-V**).
- Increased O_3 and CO_2 concentrations cause changes in interspecific competition (**II**) and consequently in the community structure (**III**).
- Legumes have an important role in interspecific competition and in the communities through their ability to fix atmospheric N (**II, III**). The fraction of legumes is expected to decrease under elevated O_3 and to increase under elevated CO_2.
- Carbon dioxide ameliorates the negative effects of O_3 (**II-V**).
- The availability of light and nutrients, and competition, are important determinants of growth and resource allocation (**I**).

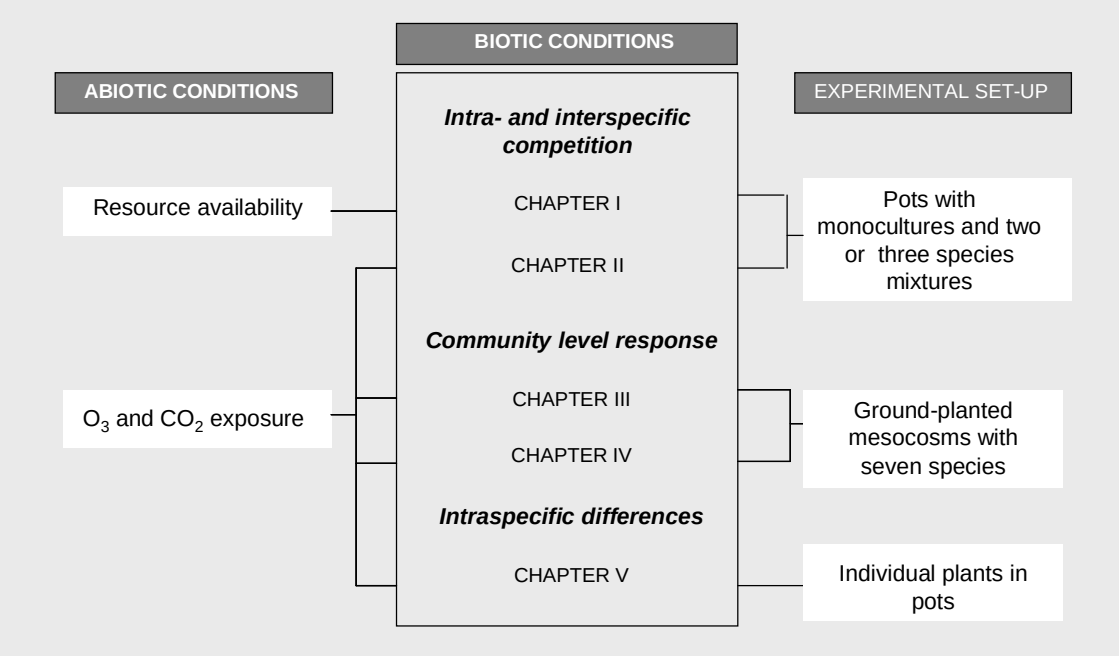

Figure 1. Relationships between the chapters.

Materials and Methods

The thesis project consisted of three O_3 and CO_2 exposure experiments (**II-V**) that were conducted as open-top-chamber (OTC) studies at Jokioinen, SW Finland, and, a smaller-scale experiment (**I**) with different availabilities of resources in greenhouses in Helsinki (Fig. 1). The OTC experiments included a competition experiment with two- and three-wise interactions (**II**), a mesocosm-scale meadow community with a large number of species (**III, IV**), and a pot experiment that assessed intraspecific differences of brown knapweed ecotypes (**V**).

1. Study biotope and species

All nine species (*Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Centaurea jacea* L., *Fragaria vesca* L., *Campanula rotundifolia* L., *Ranunculus acris* L., *Trifolium medium* L., *Lathyrus pratensis* L., *Vicia cracca* L.; Hämet-Ahti et al., 1998) used in the studies are perennials and typical to a biotope called lowland hay meadow, but they are also common to other biotopes

such as road margins and forest edges. A lowland hay meadow is a biotope listed in EU council directive 92, and it is of high conservation value. Lowland hay meadows are mainly mesic, sometimes dry meadows that are usually cut once or twice after the grasses have flowered (Pykälä et al., 1994). The biotope is also characterized by a high number of flowering plants making it an important habitat for butterflies and other invertebrates. Meadow species typically favor habitats with N-limited and slightly alkaline soil and high availability of light (Pykälä, 2001). The major threats to the biotope are overgrowth and nutrient enrichment, but several species belonging to this biotope have also been reported to be rather sensitive to O_3 (Power and Ashmore, 2002). In general, the land area covered by meadows in Finland has decreased dramatically from the 1800s towards the 2000s mainly due to changes in cattle raising. During the last meadow inventory in year 1973, the land area was only 38 400 hectares (Pykälä, 2001). The study species were representatives of three functional groups: grasses (*A. capillaris, A. odoratum*), herbs (*C. jacea, F. vesca, C. rotundifolia, R. acris*) and legumes (*L. pratensis, T. medium, V. cracca*). According to previous studies conducted on individual plants or monocultures, in general, species belonging to the functional type "grasses" have been classified as O_3-tolerant and species belonging to the functional type "legumes" as O_3-sensitive (Ashmore et al., 1996; Mortensen, 1992, 1994; Power and Ashmore, 2002). The seedlings for all the experiments were grown from seeds obtained from a commercial supplier (Kukkiva Niitty), Botanical Institutes and an ICP coordination centre. The seeds obtained from a commercial supplier were collected from natural populations in south-western Finland.

2. Competition experiment on different availabilities of light and nutrients (I)

This experiment (**I**) was established to investigate the role of varying nutrients and light and competition on the growth and allocation of two of the species (*A. capillaris* and *R. acris*) used in the O_3 and CO_2 studies. The experiment was conducted in the greenhouses of the University of Helsinki (60°13'N, 25°12'E) in the spring of 2003.

The experiment was conducted by using $9 \times 9 \times 10$ cm pots that contained 450 ml of peat and sand (1:4). Each pot received either one or two individuals of *A. capillaris* or *R. acris*, giving non-competing and intraspecific competition treatments, or one individual of both species, to provide interspecific competition treatment. The plants were watered daily by hand as needed and the excess water was allowed to drain freely. To assure adequate growth, a very mild fertilizer was given once a week.

Two independent light levels (low light and high light) were established by placing shade cloths over wooden frames. The cloth reduced the light to approximately 50 % of the high light treatment. In addition, two independent nutrient levels (low nutrients and high nutrients) were

established. The high nutrient treatment pots received 2.25 g of osmocote, a slow-release fertilizer (NPK 15-4-10), prior to plant transplantation. No osmocote was added to the low nutrient treatment. The pots with high and low nutrients were divided equally and randomly within the high and low light levels, resulting in a full-factorial design with respect to resource availability. The resource treatments and competitive situations (single individual, two conspecific individuals, or two heterospecific individuals) were combined according to a completely randomized full-factorial design, where each pot was an experimental unit.

The initial plant biomass per species was determined before the onset of the experiment. Study plants were harvested 14 times during a 48-day period. Each time one set of growth treatments was removed from each resource treatment, which resulted in 20 pots per harvest. At the end of the experiment, a final harvest was conducted on the remaining ten sets of plants. At each harvest the plants were removed from the pots and the roots were carefully separated and washed. Each plant was dried to a constant weight at 60°C for at least 48 hours, after which the root and shoot dry mass were determined for each plant. To compare the effects of resource and competition treatments, root-to-shoot ratios and total biomasses were calculated for each individual. For intraspecific competition a pot-based average was calculated.

3. OTC studies on the effects of O_3 and CO_2 (II-V)

3.1. The treatments

All the O_3 and CO_2 studies were conducted in open-top chambers (OTC) in Jokioinen (60°49'N, 23°28'E) in Southwestern Finland, at 100 m above sea level. The OTC treatments were as follows (3 replicates in each): NF (non-filtered ambient air), NF+O_3 (1.5 x ambient O_3), NF+CO_2 (1.3 x ambient CO_2) and NF+O_3+CO_2 (1.5 x ambient O_3 and 1.3 x ambient CO_2). Three open-field plots (AA) served as controls for the chamber effect, except for the experiment presented in chapter (**II**). The O_3 and CO_2 concentrations were chosen to simulate the predicted ambient concentrations in the year 2050, with a yearly increase of 0.5–2% in O_3 (Vingarzan, 2004) and a moderate 0.5% increase in CO_2 (IPCC, 2001). The plants were fumigated between 10 a.m. and 7 p.m. seven days a week, except on days with heavy rain and ambient O_3 concentrations below 20 ppb. In 2002, the exposure lasted from July 1 to August 28; in 2003, from June 3 to August 31; and in 2004, from May 18 to August 22.

OTCs (3 m in diameter, 2.8 m in height) with an added frustum were used. All the OTCs were equipped with blowers to exchange three air volumes per minute. The blowers were on for 24 hours per day. The OTCs were placed in the experimental field in a completely randomized

design. The gases were monitored at approximately 1 m above the soil surface, and in July 2004 ambient O_3 concentrations were also measured from a three-meter-high mast. Relative air humidity and temperature were measured from two NF OTCs and one AA plot.

3.2. Three-year pot experiment on plant competition (II)

To study the role of O_3 and CO_2 on plant morphology and competitive interactions, a long-term (2002-2004) pot experiment with seven different grass-herb-legume associations was designed. The chosen species were *Agrostis capillaris*, *Ranunculus acris* and *Lathyrus pratensis*. Because legumes have been proven to be responsive to O_3 and CO_2 and they have the ability to fix atmospheric nitrogen, specific interest was focused in the role of *L. pratensis*.

Each growth pot (V = 7.5 l; d = 26 cm) consisted of six plants: monocultures and the following mixtures (*L. pratensis* and *A. capillaris* 1:1, *L. pratensis* and *R. acris* 1:1, *R. acris* and *A. capillaris* 1:1, *L. pratensis*, *A. capillaris* and *R. acris* 1:1:1). Initial plant density in each pot was 113 plants/m^2. The soil in the pots was a mixture of sand and low-fertility peat (1:1), and the pots were watered with tap water when needed. Fertilizers were added only in the first summer to assure growth onset. The soil was kept intentionally N low so that the effects of N_2 fixation by *L. pratensis* would be distinguishable. In early June 2003 the pots were replaced with new, larger pots (V = 15 l; d = 33 cm) to prevent size from becoming a stress and limiting factor for plant and root growth.

Two pots per culture were randomly allocated to different OTC treatments (3 replicates in each), and non-destructive plant measurements (Table 1) were conducted in August 2002-2004. In August 2003 one set of pots was destructively harvested, and the other set of pots was harvested in August 2004.

3.3. Mesocosm study (III, IV)

To study the effects of O_3 and CO_2 on lowland hay meadow species and the community, ground-planted mesocosms with seven species were established in early June 2002. Twenty-five seedlings of *F. vesca, C. rotundifolia, R. acris, A. odoratum,* and *A. capillaris,* and five *T. medium* and eight *V. cracca* seedlings were randomly transplanted to mesocosms of approximately 2.25 m^2 (for illustrations, see Kanerva et al., 2005). The soil of the mesocosms was a peat-sand mixture and the N concentration was low compared to intact natural meadows (for details, see Kanerva et al., 2005). Each mesocosm was surrounded by a semi-transparent net to restrict expansion of the plants. The plants were watered when needed (for details, see chapter

III). The mesocosms were exposed to O_3 and CO_2 alone and in combination for three consecutive summers in 2002- 2004. The measured variables are summarized in Table 1.

3.4. Intraspecific differences in O_3 sensitivity (V)

To differentiate the role between the sensitivity of the Northern ecotypes and the (Finnish) climatic conditions potentially favourable to O_3 uptake, an experiment assessing the intraspecific differences of ecotypes originating from two different countries in Europe was designed (**V**).

A pot-based experiment with four different singly-grown ecotypes of brown knapweed (*Centaurea jacea* L.) was run in the summer of 2003. *C. jacea* was selected because it has previously been reported to be rather sensitive to O_3, has shown large intraspecific variation (Bassin et al., 2004) and has been proposed as a bioindicator for tropospheric O_3 (Bungener et al., 1999a, 2003). The two Southern Finnish populations originated from Korppoo (coastal, 61°11'N, 21°38'E) and Sälinkää (inland, 60°43'N, 25°14'E), and the two Swiss ones originated from Neuchâtel (47°00'N, 06°58'E). One individual per ecotype was transplanted to a 7.5 liter pot (26 cm ø) containing an equal mixture of peat, sand and local soil (1:1:1). Before the onset of the experiment, 10 individuals per type were destructively harvested to obtain the mean initial above-ground biomass of the seedlings. All the seedlings were watered with tap water when necessary. The plants were allowed to grow under the treatments until the end of August. The measured variables are summarized in Table 1.

4. Statistical analyses

Statistical analyses of the OTC studies (**II-V**) were conducted by using factor-ANOVA and one-way ANOVA (SPSS 12.0). The one-way ANOVA analysis was conducted in a similar manner for all the treatments, including both controls (NF and AA) in all the experiments. However, the factor-ANOVA analysis varied between experiments. In chapters **II-IV** the treatment effect was analyzed without AA plots to obtain a clearer picture of the O_3 and CO_2 effects, and the treatments were analyzed as such. In chapter **V**, however, the different treatment variables (O_3, CO_2 and OTC) were analyzed separately, and the data from the AA plots was used to assess the impact of the OTCs. Repeated-measures-ANOVA was used in assessing time-repeated measurements such as visible injuries, senescence and flowering. Tests of least significant differences (LSD) were conducted for treatment effects (**II-V**), and Tukey's post hoc test was used to assess the differences between different ecotypes (**V**). If the assumptions of normality and homogeneity of variances were not met, the data was $\log_{10}$ transformed or analyzed with a non-parametric Kruskall – Wallis test. Spearman's correlations were performed to observe possible

correlations between different variables (**III-V**). The results were considered significant at $P < 0.05$ and marginally significant at $P < 0.10$.

The analyses for chapter (**I**) were conducted with a factor-ANOVA model, where the treatments and sampling day were fixed factors. The individuals harvested from different experimental treatments at the end of the experiment were analyzed by comparing the 95% confidence intervals of the $\log_{10}$ transformed estimates, obtained from a least-squares fitting.

Results and Discussion

1. Moderate exposure and interannual variation in climate

The three summers varied significantly with respect to both climate and exposure (**III**, Finnish Meteorological Institute, 2004). The summer of 2002 was the warmest and driest, and the summer of 2004 was the coolest and wettest. Ambient one-hour mean O_3 concentrations at the top of the canopy (1 m height) during the exposures (10 a.m. to 7 p.m.) were generally below 30 ppb in the treatments receiving ambient O_3, and between 40 and 50 ppb in the treatments receiving supplemental O_3 (NF+O_3 and NF+O_3+CO_2). Daily 1-h maximum O_3 concentrations between the three years (2002-2004) varied within 74-98 ppb in the treatments receiving supplemental O_3 and within 49-67 ppb in the treatments with ambient O_3. Accumulated exposures above a threshold value of 40 ppb (AOT40) ranged from 85 to 674 in the ambient O_3 treatments and from 3 132 to 10 331 ppb h in the treatments receiving supplemental O_3 (NF+O_3 and NF+O_3+CO_2), resulting in a more than two-fold AOT40 value during the last growing season. The cumulative O_3 exposure AOT40 values of all three summers in the NF+O_3 and NF+O_3+CO_2 treatments exceeded the current critical level (3 000 ppb h over a three-month period) for protecting semi-natural vegetation as proposed by the UN-ECE convention (Karlsson et al., 2003), but they were generally below the values measured in O_3 enhancement studies in Central Europe (e.g. Power and Ashmore, 2002; Tonneijck et al., 2004). The CO_2 concentrations in the NF+CO_2 and NF+O_3+CO_2 treatments were elevated by approximately 100 ppm, which is lower than the concentrations generally used in CO_2 enhancement studies (e.g. Jongen and Jones, 1998; Marissink et al., 2002), and the daily exposure period was only nine hours.

Table 1. A summary of the measured variables, observed responses (obs) and the responsive species (sp; 1-9) to OTC, O_3, CO_2 and O_3+CO_2. The chapters (**II-V**) in which the variables were studied are also presented. Symbols indicate the direction of the significant response [p<0.05; One-way ANOVA; + increase; - decrease; 0= no response]. Parenthesis indicate p<0.1 obtained from one-way ANOVA or results p<0.05 obtained by Factor-ANOVA [for details see chapters (**II-V**)]. Symbols may indicate that only one species showed response or that several species showed the same response.

Measured variable	Chapter	OTC[a]		O_3		CO_2		O_3+CO_2	
		obs	sp.	obs	sp.	obs	sp.	obs	sp.
Species									
Photosynthesis	V	0		0		(+)	8	0	
Stomatal conductance	V	0		(-)	8	0		0	
Visible injuries	II	nm		0		0		0	
	III, V	0		+	6,7,8	0		+	6,7,8
Senescence (purple discoloration)	V	0		+		0		0	
Relative chlorophyll meter values	IV	0		-	4	0		-	4
	V	0		0		0		0	
N concentration of individual species	III	-	4	0		-	7	-	7
"Leaf area"	II, V	0		0		0		0	
Number of leaves and aerial shoots	V	0		-		+		0	
	II	nm		(+)	9	(+)	9	(+)	9
Height of shoots	II	nm		0		+	9	0	
	V	0		0		0		0	
Flowers per dry weight	IV	0		0		+	3,4	+	3,4
Onset of flowering	IV	0		-	3,7	0		-, 0	3,7
Biomass of individual species	III	+	1,3,4,7	-	3,4,(7)	0		-	3, 4, (7)
	II	nm		0		+	9	0	
	V	+		0		0		0	
Number of flowering individuals	IV	0		(-)	3	0		0	
	V	0		0		0		0	
	II	nm		0		0		0	
Number of flowers per individual	IV	0		-	3	0		-	3
	II	nm		(-)	5	(-)	5	0	
	V	0		0		0		0	
Berry fresh weight	IV	0		-	4	0		(-)	4
Community									
Early season plant coverage	IV	+		-		0		0	
Total community biomass	II	0		0		0		0	
	III	+		-		0		(-)	
Species frequencies	II	nm		0		0		0	
	III	+	1	-	3	0		-	3
Community root biomass	III	0		0		0		0	
Total community N pool	III	(+)		(-)		0		(-)	
Total number of flowers	IV	+		-		0		0	

nm = not measured; 1 = *A. capillaris* 2 = *A. odoratum* 3 = *C. rotundifolia* 4 = *F. vesca* 5 = *R. acris* 6 = *T. medium* 7 = *V. cracca* 8 = *C. jacea* 9 = *L. pratensis*

2. Lowland hay meadows: an O_3-sensitive biotope in a Northern European climate

The O_3 concentrations used were moderate and yet, six out of nine species (*Campanula rotundifolia*, *Centaurea jacea*, *Fragaria vesca*, *Ranunculus acris*, *Trifolium medium*, *Vicia cracca*) showed either significant reductions in above-ground biomass (**III**) or reproductive development (**II, IV**), visible foliar O_3 injury (**IV, V**) or both as a response to elevated O_3 (Table 1). All these responses have also previously been reported with wild plants (Black et al., 2000; Davison and Barnes, 1998). The total above-ground biomass of the mesocosms was reduced by 30 % in 2003 and by 40 % in 2004 under elevated O_3, and reductions in the early- season plant coverage of the communities were seen already after one summer of exposure (with an average AOT40 value of 5260 ppb h). Ozone exposure may reduce root and stolon biomass and consequently lower the over-wintering capacity of the plants (Davison and Barnes, 1998; Wilbourn et al., 1995). Plants that have already experienced O_3 stress during the previous summer might be more susceptible to O_3 exposure in the spring (Franzaring et al., 1999) resulting in reduced growth. However, no reductions in root biomass were recorded in the mesocosms, but this may be linked to the bulk sampling method used in the present study (**III**). In addition to early-season growth onset, an annually growing difference in above-ground biomass between the NF+O_3 and NF treatments was recorded. These observations suggest accumulation of the effects of O_3 (carry-over), which was also recently recorded in an alpine pastureland (Volk et al., 2006). However, the annually growing reductions in the biomass in the NF+O_3 treatments may also be linked to the decreased plant community N pool (**III**) and reductions in the mesocosm soil's mineral N (-35% in the NF+O_3 treatment compared with the NF treatment), reported in Manninen et al. (2005) and Kanerva et al. (in press). The reductions in mineral N might have enhanced the negative effects of O_3, as suggested by Pääkkönen et al. (1995) and Whitfield et al. (1998).

These observations support Power and Ashmore (2002), who found species typical to fens and fen-meadows to be particularly sensitive to O_3. Furthermore, the conditions in Finland seem particularly favourable to O_3 uptake, because the concentrations used were generally below those used in previous studies (e.g. Power and Ashmore, 2002; Tonneijck et al., 2004), and the Swiss *C. jacea* ecotypes showed extensive visible injuries at low O_3 concentrations compared with the studies conducted in Central Europe (**V**; Bassin et al., 2004). Possible explanations for the responsiveness of the plants in the present studies may be attributed to the high relative air humidity (Benton et al., 2000; Krupa et al., 1995) and long summer days (De Temmerman et al., 2002) that may cause plants to keep their stomata open for long period of time and thus increase O_3 damage. A simulation by Embersson et al. (2000) did indeed show that the highest O_3 fluxes occur in Northern Europe, as opposed to the areas with the highest O_3 concentrations. However, when interpreting these results concerning biotope sensitivity, it is important to recognize that

the mesocosm communities were newly established and that O_3 responses are usually more pronounced in younger communities than in older already established communities (Fuhrer et al., 2005).

3. Intra- and interspecific differences in O_3 sensitivity

The intensity of O_3-induced growth losses and visible injuries varied between different species (**III, IV**; Table 1) and ecotypes (**V**), as previously reported by Bassin et al. (2004), Pearson et al. (1996) and Black et al. (2000). The visible O_3 injuries and reductions in growth were not correlated with each other. The legume *T. medium* exhibited visible injuries in the summer of 2004 (**III**), but showed no significant reductions in biomass or flower production (**III, IV**). Similarly, the *C. jacea* ecotypes, which all showed extensive visible injuries, did not experience O_3-induced growth reductions (**V**). These observations are in line with the previous studies that have shown that visible injuries are not always correlated with measurable effects on growth (Bender et al., 2006; Bergmann et al., 1995; Davison and Barnes, 1998). The sensitivity of legumes (*T. medium* and *V. cracca*) and herbaceous *C. jacea* in terms of visible O_3 injuries has been widely recognized in the literature (Bungener et al, 1999a, 2003; Davison and Barnes, 1998; Power and Ashmore, 2002). *C. jacea* was the species most sensitive to O_3 in terms of occurrence of visible injuries. In the summer of 2003, with an AOT40 value of less than 5000 ppb h, visible injuries were recorded in all the *C. jacea* individuals, but not in any of the species in the mesocosm. Visible injuries appeared in the legumes (*T. medium* and *V. cracca*) only in the growing season of 2004, which can be explained in more ways than one. The exposure level during the summer of 2004 was significantly higher than that of the two previous summers (approximately 10 000 ppb h), and the occurrence of high peak values early in the growing season may have enhanced the emergence of visible injuries (Pihl Karlsson et al., 1995; Timonen et al., 2004). Additionally, the exceptionally wet summer of 2004 may have increased O_3 uptake, hence the development of visible injuries (Benton et al., 2000).

The most sensitive functional group in terms of growth reduction and reproduction was the group of small non-leguminous herbs, especially *C. rotundifolia*, which showed reduced biomass production and decreases in flower production in the summers of 2003 and 2004 (**III-IV**). Also *F. vesca* reduced its above-ground biomass in the summer of 2003 (**III),** and the fresh berry weights of *F. vesca* were smaller in the NF+O_3 treatment compared with those in the NF treatment in the summer of 2004 (**IV**). Unlike previous studies on other leguminous species (Ashmore and Ainsworth, 1995; Ashmore et al., 1996; Gimeno et al., 2004), *T. medium*, *V. cracca* and *L. pratensis* did not prove to be a particularly O_3-sensitive species with regard to growth reductions (**II-IV**).

The experiment with four different ecotypes of *C. jacea* (**V**) revealed that the proportion of O_3-specific visible injuries was highest in the Swiss plants. Visible injuries correlated positively with early flowering, high dry matter production and high stem dry weight (**V**). This confirms the observations by Bassin et al. (2004) that flowering is associated with high sensitivity in *C. jacea* types. According to Bassin et al. (2004), this could be attributed to changes in resource allocation or changes in physical and micro-meteorological conditions. Similar O_3 sensitivity at the onset of flowering has been observed in *Arabidopsis thaliana* L., and it was explained by the plant's lowered capacity to detoxify oxidative stress in the leaves (Ye et al., 2000). The high relative growth rates and faster (phenological) development of the Swiss ecotypes explain their earlier senescence manifested as a higher percentage of leaves with purple pigmentation and lower SPAD values. According to Schenk (2003), the O_3 sensitivity of *C. jacea* types is related to early senescence, as antioxidants decrease along with age, making senescing plants more susceptible to stress factors, such as air pollutants (Larcher, 2003; Pell et al., 1997). The Finnish ecotypes required a longer time to shift into the reproductive stage, which is in accordance with the observations on the Norwegian type in the study by Bassin et al. (2004).

Assessing the factors behind the O_3 sensitivity of the mesocosm species was not as straightforward (**III**, **IV**). Neither functional group nor reproductive strategy explained the sensitivity. There are, however, several factors that link the most sensitive species, *C. rotundifolia* and *F. vesca*. These plants are relatively small and grow close to the bottom of the canopy. In natural conditions, O_3 concentrations are vertically distributed so that the highest concentrations are measured near the top of the canopy and the lowest at the bottom of the canopy (Davison et al., 2003; Fuhrer et al., 2005). According to Finkelstein et al. (2004), O_3 concentrations at a height of 20 cm can be roughly one-half of those measured at a height of 1 meter. However, in the OTCs the air was circulated and the concentrations were equally high in all parts of the canopy. It could therefore be hypothesized that the reason behind the sensitivity of *C. rotundifolia* and *F. vesca* was indeed their previous acclimation to lower O_3 concentrations due to natural stratification. This sort of correlation between O_3 sensitivity and a lack of previous exposure has been previously reported with several ecotypes originating from different geographical locations (Manninen et al., 2003; Pearsson et al., 1996; Whitfield et al., 1997). The reduced light availability and possibly lower vapour pressure deficit at the bottom of the canopy might also increase O_3 uptake. A reduction in the availability of light due to increased competition may therefore increase the O_3 sensitivity of certain lower-canopy species in the communities (Keelan et al., 2005). In addition, the biomasses of both species in the mesocosm were low, and the N concentrations (1.0% dry weight on average) were lower than generally measured in other grassland forbs (1.2 and 1.95% dry weight; Marissink et al., 2002; Reich et al., 2001) indicating that they also are weak competitors for nutrients. The N concentrations of grasses and legumes were comparable to or higher (**III**) than those reported in the literature (Marissink et al., 2002; Reich et al., 2001).

4. The role of competition and community-level responses to O_3

The experiment with different resource availabilities and competition (**I**) revealed that nutrients were the most determinant factor on the growth of both *A. capillaris* and *R. acris* (**I**). This is in agreement with Weiner and Thomas (1986), who showed that nutrients determine growth, while light determines size differences among competing plants. In general, light had a stronger role in determining biomass accumulation than competition, but at the end of the experiment competition reduced the biomass accumulation of *R. acris* more than light. *R. acris* and *A. capillaris* were highly plastic and allocated their growth in qualitative agreement with the optimal allocation theory, i.e., low intensity of light increased shoot allocation, while low nutrients increased root allocation (**I**, Bloom et al., 1985). In general, both intra- and interspecific competition reduced biomass accumulation in both species when measured at the end of the experiment. Similarly, under low light conditions competition increased relative biomass allocation to roots, which may reflect stronger competition for nutrients than light. The largest reductions in growth by interspecific competition were measured from the high light, low nutrient treatment, which contradicts Grime's theory (1979), where competition is predicted to be more intense at high nutrient levels. *R. acris* was a better competitor than *A. capillaris* in the all treatments except for the high light, high nutrients treatments.

Increased O_3 and CO_2 concentrations did not, however, cause changes in the community structure (**III**) or in interspecific competition (**II**), as expected according to the literature (Andersen et al., 2001; Ashmore and Ainsworth, 1995; Ashmore et al., 1995; Barbo et al., 1998, 2002). The proportion of legumes was not altered by O_3 or CO_2 exposures in either the mesocosm (**III**) or the pot competition (**II**) experiment.

The fraction of functional types in the mesocosms remained unchanged, irrespective of the fumigation (**III**). However, the most sensitive species in terms of vegetative growth and reproduction, *C. rotundifolia* and *F. vesca*, were also the least abundant species irrespective of the treatment (**III, IV**). This might indicate O_3-induced suppression of the weakest competitors. Similarly, the most abundant *T. medium* and *A. capillaris*, did not show growth reductions. In addition to reductions in growth, *C. rotundifolia* decreased flower production (**IV**) and the berry weight of *F. vesca* was reduced (**IV**). The cumulative effects of decreased biomass production and reductions in the number of flowering individuals and flowers of *C. rotundifolia* (**IV**) might potentially be a very pernicious combination in terms of the success of the species in the community. On the other hand, strawberry has a very effective way of complementing sexual reproduction by vegetative reproduction through runners (Hämet-Ahti et al., 1998), which could compensate for the decreases in sexual reproduction observed in the present study. Supporting this, initial reduction in 2003 and following compensation in 2004 were observed in the above-ground biomass of *F. vesca*. This may be explained as physiological and morphological

adaptation (Bungener et al., 1999b), but alternatively it may be attributed to weaker competition in the elevated O_3 treatments. Wild strawberries thrive in sunny spots, and their fast vegetative growth can benefit from reductions in total community coverage, and thus reduced competition.

Ozone had no effect on growth or the formation of visible injuries in the studied species in the pot competition experiment (**II**), and therefore the species mixture was irrelevant. Similar results were recently reported from pot studies by Tonneijck et al. (2004) and Bender et al. (2006). The lack of growth responses in the competition experiment (**II**) may be explained by the species used, and possibly by the N poor soil. Although *R. acris* and *A. capillaris* showed significant plasticity in growth and allocation under variable availabilities of light and nutrients (**I**), these species were not particularly sensitive to O_3 in either the pot or the mesocosm experiment (**II-IV**). The soil used for the mesocosm (**III, IV**) and competition (**II**) experiments was initially very low N compared with the concentrations measured in natural meadows (Kanerva et al., 2005), and it is likely that N limitation restricted the growth of *R. acris* and *A. capillaris*, in particular. Contrary to experiment (**I**), *R. acris* was found to be the weakest competitor in the pot competition experiment (**II**). Although O_3 did not decrease the growth of leguminous *L. pratensis* (**II**), competition irrespective of fumigation did suggest that the presence of legumes in species mixtures in low N habitats may be beneficial. The mean biomass of *R. acris* and *A. capillaris* was increased in the summer of 2004 when grown in the presence of *L. pratensis* (**II**) compared with those grown without the legume. It seems that the plants became N limited towards the end of the experiment. Interspecific competition in the pots in the OTC experiment was less intense in 2003 as mean individual above-ground biomass of *R. acris* and *A. capillaris* were higher (0.9 g and 2.1 g dw, respectively; **II**) than in the same species in the greenhouse experiment (**I**) under low nutrients and high light (0.5 g dw, in both species; data not presented). However, as the OTC experiment (**II**) proceeded, nutrients (most likely N) became limiting and the mean biomass per original individuals was reduced to 0.1g in *R. acris* and 0.9 g in *A. capillaris*.

In contrast to the pot experiment, the biomass in the mesocosms kept on increasing year by year, indicating that this community was not as nutrient stressed (**III**). The mesocosm communities were rich in legumes and they seemed to fertilize the whole community, as the soil mineral N increased from the fall of 2002 to the fall of 2004 (Kanerva et al., in press). The mean mineral N concentration in the NF treatment, for example, was 1.84 μg min N g^{-1} dw^{-1} in the fall of 2002 and 4.60 μg min N g^{-1} dw in the fall of 2004 (Kanerva et al., in press). However, the soil mineral N concentrations in 2004 were still well below those measured in three intact meadows with similar species coverage [5.05 - 18.53 μg min N g^{-1} dw^{-1}; Kanerva et al. (2005)]. The mesocosms were developing communities, and it is possible that in the long run, increases in the N concentration might lead to significant changes in the community structure and possibly in the O_3 sensitivity of the community, as well.

5. CO_2 alone induced only minor responses

Generally, the effects of CO_2 were minor (Table 1). Carbon dioxide tended to increase the photosynthesis and leaf number of *C. jacea* (**V**), height and biomass production in *L. pratensis* (**II**), and the number of flowers per dry weight in *C. rotundifolia* and *F. vesca* (**IV**), but these changes were slight and restricted to certain species and experiments. Additionally, the N concentration of *V. cracca* was reduced under elevated CO_2 (**III**). The responses were consistent with those reported in the previous studies (Bazzaz, 1990; Marissink et al., 2002; Pritchard et al., 1999; Reich et al., 2001), although according to the literature stronger responses were expected (e.g. Ainsworth and Long, 2005; Bazzaz, 1990; Jablonski et al., 2002; Jongen and Jones, 1998; Lüscher and Nösberger, 1997). For instance, *A. capillaris* has previously been reported to enhance growth rather markedly (from 17% to 56% and 100% increase in whole plant dry weight) under elevated CO_2 (Baxter et al., 1994; Jongen and Jones, 1998; Newbery et al., 1994), but no such stimulations were recorded in the present studies (**II, III**). In addition, CO_2-induced growth enhancements above- and below-ground, were altogether lacking from the mesocosms (**III, IV**), and no changes in the species frequencies were recorded (**III**), as was expected according to the literature (Bazzaz, 1990; Owensby et al., 1999; Poorter and Navas, 2003). However, there are several explanations that might enlighten the moderate responses. Firstly, the CO_2 concentration was increased by only 100 ppm, whereas several studies have doubled the CO_2 concentrations (e.g., Jongen and Jones, 1998; Marissink et al., 2002). However, these concentrations were realistic with respect to those projected for the year 2050 (IPCC, 2001). In addition, the daily exposure period was only nine hours. Furthermore, as stated previously, the soil used for the pot competition and mesocosm experiments was initially N poor (Kanerva et al., 2005; Manninen et al., 2005), and the plants may not have been able to benefit from the augmentation of CO_2 (Poorter and Pérez-Soba, 2001). However, this is not supported by the fact that the growth or relative abundance of N_2-fixing legumes did not increase in the mesocosms.

6. Only slight amelioration of O_3 damage by elevated CO_2

Previous studies have shown that CO_2 may ameliorate negative O_3 responses (e.g. Booker and Fiscus, 2005; Booker et al., 2005, CardosoVilhena et al., 2004; Fiscus et al., 2005; Heagle et al., 1999,2000; McKee et al., 1997, 2000; Morgan et al., 2003; Mulholland et al., 1997a, 1997b; Volin et al., 1998), while others have shown that CO_2 did not protect against O_3 (Balaguer et al., 1995; Heagle et al., 2002, 2003; Kull et al.,1996). In the present studies, in general, CO_2 did not ameliorate the negative effects of O_3 on the formation of visible injuries and relative chlorophyll meter values, the biomass and the number of flowers per individual species (Table 1). CO_2 slightly ameliorated O_3-induced reductions in early-season plant coverage (**IV**), total community biomass (**III**) and the total number of flowers (**IV**) in the mesocosms. Elevated O_3 did not reduce

the beneficial effects of CO_2 on *L. pratensis* height growth (**II**). In the *C. jacea* experiment, the capacity of CO_2 to ameliorate the specific visible O_3 injuries depended on the ecotype (**V**). Significant amelioration took place only in the most O_3 -sensitive ecotype, and the least sensitive ecotypes exhibited more O_3-specific injuries when O_3 and CO_2 were given in combination, resulting in diminished differences between the different ecotypes. This is in agreement with a study on two *Trifolium repens* L. clones (Heagle et al., 1993), but contrary to a study by Dickinson et al. (2001), who studied two trembling aspen clones and noticed that the addition of CO_2 to O_3 exposure only counteracted the negative impact of O_3 in the tolerant clone. An opposite trend was recorded for non-specific reddening. When exposed to the NF+O_3+CO_2 treatment, the Finnish ecotypes hardly showed any reddening, whereas the individuals of the Swiss ecotypes exhibited extensive reddening. It may be speculated that CO_2 delayed O_3-related senescence in the Finnish ecotypes. There also seemed to be species- and variable-specific differences in CO_2 amelioration of the effects of O_3 (Table 1). For instance, the height growth of *L. pratensis* (**II**) and the N concentration of *V. cracca* (**III**) were similar in the NF+CO_2 and NF+O_3+CO_2 treatments, whereas no amelioration was observed in the growth reductions of *C. rotundifolia* and *F. vesca* in the NF+O_3 and NF+O_3+CO_2 treatments (**III**). Similarly, no CO_2 amelioration was recorded in the number of flowers per *C. rotundifolia* individuals per se (**IV**), but the flowers per dry weight index (**IV**) was increased in both of the NF+CO_2 and NF+O_3+CO_2 treatments.

It has been suggested that low availability of nutrients may increase species sensitivity to O_3 (Fuhrer et al., 2005; Pääkkönen et al., 1995; Whitfield et al., 1998) and may reduce the beneficial effects of CO_2 (Poorter and Navas, 2003). Previous experiments with a combination of O_3 and CO_2 have mainly been conducted on crops, which usually grow under heavy fertilization. The slight amelioration observed in the present studies, compared with those of extensive amelioration in crops, might be linked to the availability of N. It may be speculated that CO_2 amelioration of the negative effects of O_3 would be less under low than under high N soil. The lack of compensation in individual species also may be connected to the low CO_2 concentrations. Heagle et al. (1993) studied *Trifolium repens* and noticed that only the highest CO_2 concentration used (710 ppm) protected clover from the effects of tropospheric O_3. Some studies have reported that elevated CO_2 may indeed enhance the detrimental effects of O_3 on photosynthesis and growth (Johnson et al., 1996; Kull et al., 1996; Wustman et al., 2001). Based on the present studies, most of the phytotoxic effects of tropospheric O_3 are not diminished by a moderate increase in CO_2 under low N availability.

7. OTCs increased plant growth

Apart from the O_3 and CO_2 effects, there were also rather strong chamber effects (**III-V**). The OTCs increased, although non-significantly, the temperature, decreased relative humidity, and filtered some of the ambient O_3. The OTCs increased the above-ground biomass production of several species (*A. capillaris, C. rotundifolia*, *F. vesca* and *V. cracca*) in the mesocosms (**III**; Table 1) and in the *C. jacea* experiment (**V**), an observation which is in good agreement with the earlier studies (e.g. Danielsson et al., 1999; Owensby et al., 1993, 1999; Sanders et al., 1991). In addition, the OTCs improved early-season growth onset (**IV**), enhanced the total number of produced flowers (**IV**) and increased the proportion of grasses, especially of *A. capillaris* in the mesocosms (**III**). The Finnish *C. jacea* ecotypes benefited more from the OTC conditions compared to their Swiss counterparts, which is in agreement with the observations of Danielsson et al. (1999) on *Phleum* genotypes originating from areas with different summer temperatures. The OTC effect was not analyzed in chapter (**II**) due to extensive vole damage in the AA plots, and no OTC-related changes in the reproductive development of individual species were recorded (**IV**).

8. Methodological considerations

Most of the previous studies on wild plants and O_3 have been conducted as single plant or monoculture pot studies (Davison and Barnes, 1998), although studies on competition are beginning to appear (Bender et al., 2006; Fuhrer et al., 2005; Tonneijck et al., 2004). I wanted to assess the effects of elevated O_3 and CO_2 on competition using both rather simple pot experiments and more realistic ground-planted multi-species mesocosms. Both approaches had their advantages. The pot experiments were designed to provide better control of the experimental conditions in order to get mechanistic information on the strength of intra- and interspecific competition, but they may have introduced limitations to the rooting volume. The large 2.25 m^2 multispecies mesocosms provided a more natural setting and they seemed comparable to natural meadows. The mean above-ground biomass per area unit (408 g dw m^{-2}) of the mesocosms corresponded to that of natural meadows (Marissink et al., 2002). The greenhouse gas emissions and potential microbial activities of the same mesocosms were also at the same level as those of three natural meadows (Kanerva et al., 2005), suggesting that the results obtained from the mesocosm study may well be applicable to intact meadows. The drawback of the mesocosms was the abundance of individuals, which made it quite difficult to assess the role of the studied gases on reproductive development. Additionally, as the mesocosms did not allow direct assessment of the competitive interactions, but rather reported the growth of plants under

competition, I cannot point out the influence of competition as a single cause for the potentially increased sensitivity.

While OTCs are an economic alternative because they reduce the amount of O_3 and CO_2 used in the experiment, the OTC enclosure alters the microclimate for the plants grown within. The forced ventilation of the OTCs results in the disappearance of the vertical O_3 gradient within the canopy, which is replaced by uniform bulk air at all heights of the canopy. Also the boundary layer resistance is decreased in the OTCs compared with that in the field, which may result in increased O_3 uptake (Nussbaum and Fuhrer, 2000) and may overestimate the effects of O_3 and CO_2 in the OTC studies compared with free-air enrichments conducted at same O_3 and CO_2 concentrations (Fuhrer et al., 1997; Morgan et al., 2003, 2005). However, a recent free-air fumigation (FACE) study by Morgan et al. (2006) revealed that O_3 -induced yield losses in soybeans (*Glycine max*) grown under open-air conditions were even greater than those reported in earlier chamber studies. Furthermore, the OTC enclosure may prohibit pollination, which may cause significant distortions in interpreting the effects of O_3 exposure on reproduction. An alternative, although more costly, is a free-air fumigation system (Volk et al., 2006). Irrespective of the drawback introduced by ventilation and bulk air, using OTCs does provide valuable information on the complex interactions of climate change including increases in temperature and decreases in solar radiation (Runeckles, 2002).

Conclusions

Lowland hay meadows proved to be an O_3-sensitive biotope, as the O_3 concentrations used (40-50 ppb) were moderate and yet, six out of nine species (*Campanula rotundifolia, Centaurea jacea, Fragaria vesca, Ranunculus acris, Trifolium medium, Vicia cracca*) showed either significant reductions in biomass or reproductive development, visible ozone injury or any two as a response to elevated O_3. The Finnish climate seemed to increase the species sensitivity to O_3, as the Swiss *C. jacea* ecotypes showed extensive visible O_3 injuries at lower concentrations than reported previously in Central Europe. Plant species and ecotypes exhibited large intra- and interspecific variation in their response to O_3, but the occurrence of visible injuries and reductions in growth were not correlated with each other. The species that suffered the largest reductions in growth were, however, the same that experienced reductions in the reproductive organs. Among the most sensitive species with respect to growth and reproduction was *C. rotundifolia*. The higher sensitivity of the Swiss *C. jacea* ecotypes compared with the Finnish ones was attributed to their fast (reproductive) development.

Increased O_3 and CO_2 concentrations did not cause changes in interspecific competition, and the proportion of legumes was not altered by O_3 or CO_2 exposures in either the pot competition or the mesocosm experiment. The presence of leguminous *L. pratensis* did, however, increase the growth of *A. capillaris* and *R. acris* when grown as two- or three-species mixtures. The interspecific differences in O_3 and CO_2 sensitivity did not lead to changes in community composition, but the total community biomass production was decreased up to 40% under the NF+O_3 treatment. However, the largest O_3 induced growth reductions were seen in the least abundant species (*C. rotundifolia* and *F. vesca*), which may indicate O_3-induced suppression of weak competitors. Ozone seemed to have a strong carry-over effect, which might be attributed to the altered resource allocation or decreases in the soil mineral N.

The overall effects of CO_2 were relatively small and mainly restricted to individual species and several measured variables. The lack of CO_2 responses may be attributed to the low levels of enhancement, the restricted exposure period and the N limited soil. In general, CO_2 did not ameliorate the negative effects of O_3 on formation of visible injuries and senescence, biomass and the number of flowers produced by individual species. CO_2 slightly ameliorated the negative effects of O_3 on early-season plant coverage and total community biomass. The effects of CO_2 on O_3 mitigation in *C. jacea* depended on the ecotype. Based on the present studies, most of the deleterious effects of tropospheric O_3 are not diminished by a moderate increase in CO_2 under low N availability, and variation exists between different species and variables. The OTCs increased the total above-ground biomass production both in the mesocosms and in *C. jacea* individuals. Using OTCs may contort extrapolation of the O_3 results to non-experimental conditions, e.g. due to the formation of bulk air and forced ventilation, but it does provide valuable information on the complex interactions of climate change.

Long-term predictions are difficult to make on the basis of a few fairly simply experiments, but the studies indicate that the growth of several herb species decreases with increasing atmospheric O_3 concentrations, and that these changes may pose a threat to the biodiversity of meadows. An increase in temperature may, however, compensate for some of these changes. Ozone-induced reductions in the total community biomass production and N pool are likely to have important consequences for the nutrient cycling of the ecosystem.

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